

CLAIMS

We claim:

*SABR* > 1. A composition comprising at least one recombinase and two substantially complementary single stranded targeting polynucleotides, each containing:  
5 a) at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence; and  
b) at least one anchoring sequence.

10 2. The composition of claim 1 further comprising a secondary probe, wherein said probe is substantially complementary to at least one of said anchoring sequences.

15 3. The composition of claim 1 wherein said anchoring sequences form a triplex anchor.

4. The composition of claim 1 wherein said anchoring sequences form a quadruplex anchor.

5. The composition of claim 1 wherein said anchoring sequences form a Z-DNA anchor.

20 6. The composition of claim 1 wherein said anchoring sequences form a B-DNA anchor.

*SABR* > 7. The composition of claim 1 wherein said anchoring sequences form an A-DNA anchor.

8. The composition of claim 1 wherein said anchoring sequences comprise RNA.

25 9. The composition of claim 1 wherein said anchoring sequences comprise DNA.

10. The composition of claim 1 wherein at one of said targeting polynucleotides comprises protein nucleic acid.

30 11. The composition of claim 1 wherein said anchoring sequences comprise DNA and RNA.

12. The composition of claim 1, wherein said recombinase is a species of prokaryotic recombinase.

35 13. The composition of Claim 12, wherein said prokaryotic recombinase is a species of prokaryotic RecA protein.

*SABR* > 14. The composition of Claim 12, wherein said RecA protein species is *E. coli* RecA.

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15. The composition of claim 1, wherein said recombinase is a species of eukaryotic recombinase.

16. The composition of claim 15, wherein said recombinase is a Rad51 recombinase.

17. The composition of claim 15, wherein said eukaryotic recombinase is a complex of recombinase proteins.

18. The composition of claim 1 wherein at least one of said single stranded nucleic acids contains at least one substituent.

19. The composition of claim 18 wherein said substituent is a chemical substituent.

20. The composition of claim 18 wherein said substituent is a protein.

21. The composition of claim 18 wherein said substituent is selected from the group consisting of intercalators, cross-linking moieties, labels, photoactive moieties, nucleic acid scission inducing moieties, purification moieties, and nucleic acid modification moieties.

22. A composition comprising a double D-loop comprising a target nucleic acid and two substantially complementary single stranded targeting polynucleotides, each containing:

a) at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence of said target nucleic acid; and

b) at least one anchoring sequence.

23. The composition of claim 22 further comprising a secondary probe, wherein said probe is substantially complementary to at least one of said anchoring sequences.

24. The composition of claim 22 wherein said anchoring sequences form a triplex anchor.

25. The composition of claim 22 wherein said anchoring sequences form a quadruplex anchor.

26. The composition of claim 22 wherein said anchoring sequences form a Z-DNA anchor.

27. The composition of claim 22 wherein said anchoring sequences form a B-DNA anchor.

28. The composition of claim 22 wherein said anchoring sequences form an A-DNA anchor.

29. The composition of claim 22 wherein said anchoring sequences comprise RNA.

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44. The composition of claim 43 further comprising a secondary probe, wherein said probe is substantially complementary to at least one of said anchoring sequences.

5 45. The composition of claim 43 wherein said anchoring sequences form a triplex anchor.

46. The composition of claim 43 wherein said anchoring sequences form a quadruplex anchor.

10 47. The composition of claim 43 wherein said anchoring sequences form a Z-DNA anchor.

48. The composition of claim 43 wherein said anchoring sequences form a B-DNA anchor.

15 49. The composition of claim 43 wherein said anchoring sequences form an A-DNA anchor.

50. The composition of claim 43 wherein said anchoring sequences comprise RNA.

51. The composition of claim 43 wherein said anchoring sequences comprise DNA.

20 52. The composition of claim 43 wherein at least one of said targeting polynucleotides comprises protein nucleic acid.

53. The composition of claim 43 wherein said anchoring sequences comprise DNA and RNA.

25 54. The composition of claim 43, wherein said recombinase is a species of prokaryotic recombinase.

55. The composition of Claim 54, wherein said prokaryotic recombinase is a species of prokaryotic RecA protein.

30 56. The composition of Claim 55, wherein said RecA protein species is *E. coli* RecA.

57. The composition of claim 43, wherein said recombinase is a species of eukaryotic recombinase.

35 58. The composition of claim 57, wherein said recombinase is a Rad51 recombinase.

59. The composition of claim 57, wherein said eukaryotic recombinase is a complex of recombinase proteins.

40 60. The composition of claim 43 wherein at least one of said single stranded nucleic acids contains at least one substituent.

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61. The composition of claim 60 wherein said substituent is a chemical substituent.

62. The composition of claim 60 wherein said substituent is a protein.

5 63. The composition of claim 60 wherein said substituent is selected from the group consisting of intercalators, cross-linking moieties, labels, photoactive moieties, nucleic acid scission inducing moieties, purification moieties, and nucleic acid modification moieties.

64. A cell containing the composition of claim 1, 20, or 43.

65. The cell of claim 64 which is a eukaryotic cell.

66. The cell of claim 64 which is a prokaryotic cell.

67. A method of modulating transcription or replication of a pre-selected target sequence comprising contacting said target sequence with a composition comprising at least one recombinase and two substantially complementary single stranded targeting polynucleotides each containing:

- a) at least one homology clamp that substantially corresponds to or is substantially complementary to said preselected target nucleic acid sequence; and
- b) at least one anchoring sequence; whereby, the transcription or replication of said target sequence is modulated.

68. The method of claim 67 wherein said target sequence is contained within a cell.

69. The method of claim 68 wherein said cell is a eukaryotic cell.

70. The method of claim 69 wherein said eukaryotic cell is selected from the group consisting of mammalian cells, plant cells, and yeast cells.

71. The method of claim 67 wherein said cell is a prokaryotic cell.

72. The method of claim 67 wherein at least one of said single stranded nucleic acids contains at least one substituent.

73. The method of claim 72 wherein said substituent is a chemical substituent.

74. The method of claim 73 wherein said chemical substituent is a protein.

75. The method of claim 73 wherein said chemical substituent is selected from the group consisting of intercalators, cross-linking moieties, labels, photoactive moieties, nucleic acid scission inducing moieties, purification moieties, and nucleic acid modifying moieties.

5 76. The method of claim 68, wherein said targeting polynucleotides are coated with said recombinase.

77. A method of Claim 76, wherein said recombinase is a species of prokaryotic recombinase.

10 78. The method of Claim 77, wherein said prokaryotic recombinase is a species of prokaryotic RecA protein.

79. The method of Claim 78, wherein said RecA protein species is *E. coli* RecA.

15 80. The method of claim 79, wherein said recombinase is a species of eukaryotic recombinase.

81. The method of claim 80, wherein said eukaryotic recombinase is a Rad51 recombinase.

20 82. The method of claim 80, wherein said eukaryotic recombinase is a complex of recombinase proteins.

83. The method of claim 68 wherein the expression of said pre-selected target sequence is involved in a disease state of an animal.

25 84. The method of claim 68 wherein said target sequence is a promoter sequence.

85. The method of claim 84 wherein said transcription of said promoter sequence is increased.

30 86. The method of claim 67 wherein said target sequence is an origin of replication sequence.

87. The method of claim 86 wherein said replication of said target sequence is decreased.

35 88. The method of claim 67 wherein said target sequence is a viral target sequence.

89. A method of treating a disease state of a plant or animal caused by expression of a disease gene comprising: administering to the animal a composition comprising at least one recombinase and two substantially complementary single stranded targeting polynucleotides, each containing:

40 a) at least one homology clamp that substantially corresponds to or is substantially complementary to said disease gene; and

b) at least one anchoring sequence; whereby said disease state is treated.

90. A method of detecting a double stranded nucleic acid target sequence comprising:

- adding a composition comprising at least one recombinase and two substantially complementary single stranded targeting polynucleotides, each containing:
  - at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence; and
  - at least one anchoring sequence;

to a sample containing said target sequence under conditions which allow the formation of a double-D loop; and

- detecting the presence of said double-D loop.

91. The method of claim 90 wherein said target sequence is contained within a cell.

92. The method of claim 90 wherein at least one of said single stranded nucleic acids comprises a substituent.

93. The method of claim 90 wherein said substituent is a label.

94. A method of isolating either strand of a double stranded target sequence comprising:

- adding a composition comprising at least one recombinase and two substantially complementary single stranded targeting polynucleotides, each containing:
  - at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence; and
  - at least one anchoring sequence;

to a sample containing said target sequence under conditions which allow the formation of a double-D loop; and

- isolating said double-D loop.

95. The method of claim 94 further comprising cloning said target sequence.

96. The method of claim 94 further comprising removing said targeting polynucleotides from said double-D loop.

97. The method of claim 94 further comprising sequencing all or part of said target sequence.

98. The method of claim 94 wherein at least one of said targeting polynucleotides comprises at least one substituent.

99. The method of claim 98 wherein said substituent is a purification moiety.

100. A method of isolating either strand of at least one member of a gene family comprising:

5       a) adding a composition comprising at least one recombinase and at least two substantially complementary single stranded targeting polynucleotides, each containing:

      i) at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence, wherein said pre-selected sequence is a motif shared by the members of said family; and

      ii) at least one anchoring sequence;

10      to a sample containing said target sequence under conditions which allow the formation of a double D-loop; and

      b) detecting the presence of said double D-loop; whereby said member of said gene family is isolated.

15      101. The method of claim 100 wherein more than one member of said gene family is isolated.

102. The method of claim 101 further comprising cloning said member of said gene family.

20      103. The method of claim 100 wherein at least one of said two substantially complementary single stranded targeting polynucleotides comprises at least one substituent.

104. The method of claim 103 wherein said substituent is a purification moiety.

25      105. A method of producing a transgenic non-human organism comprising:

      a) introducing into a donor nucleus at least one recombinase and two substantially complementary single stranded targeting polynucleotides, each containing:

      i) at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence; and

      ii) at least one anchoring sequence;

30       b) transplanting said nucleus into an oocyte to produce a recombinant zygote; and

      c) producing a transgenic organism from said recombinant zygote.

35      106. A method of producing a transgenic plant comprising:

      a) introducing into a zygote at least one recombinase and two substantially complementary single stranded targeting polynucleotides, each containing:

      i) at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence; and

      ii) at least one anchoring sequence;

      under conditions which allow formation of a double D-loop;

40       b) producing a transgenic plant from said zygote.

107. A method of determining the function of a double stranded nucleic acid target sequence comprising:

- adding a composition comprising at least one recombinase and two substantially complementary single stranded targeting polynucleotides each containing:
  - at least one homology clamp that substantially corresponds to or is substantially complementary to said preselected target nucleic acid sequence; and
  - at least one anchoring sequence;

5 to a cell containing said target sequence under conditions which allow the formation of a double D-loop;

- identifying an altered phenotype in said cell; whereby the function of said target sequence is determined.

108. A kit comprising at least one recombinase and two substantially complementary single stranded targeting polynucleotides, each containing:

- at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence; and
- at least one anchoring sequence.

109. A method of inhibiting double stranded nucleic acid rotation or branch migration comprising: adding a composition comprising at least one recombinase and two substantially complementary single stranded targeting polynucleotides, each containing:

- at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence; and
- at least one anchoring sequence;

20 to a sample containing said target sequence under conditions which allow the formation of a double D-loop.

110. The method of claim 109 wherein said anchoring sequence is a triplex or quadruplex anchor.

111. The method of claim 109 wherein further comprising added a secondary probe, wherein said probe is substantially complementary to said anchoring sequence.

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112. A composition comprising a double D-loop comprising a target nucleic acid and two substantially complementary single stranded targeting polynucleotides, each containing:

- at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence of said target nucleic acid;
- at least one anchoring sequence; wherein said anchoring sequence forms an anchoring structure

35 and a protein bound to said anchoring structure.

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